

An Experimental Enquiry into the Factors which Determine the Growth and Activity of the Mammary Glands

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Growth and Activity of the Mammary Glands.

505

DESCRIPTION OF PLATE.

All the figures have been drawn under an oil immersion, 2 mm. obj. No. 6.

- FIGS. 1, 2, 3.—Sections of *Eupomatus* egg. Fixed in Flemming. $\times 600$.
 „ 4.—Section of *Eupomatus* egg. Fixed in Hermann solution. $\times 600$.
 „ 5.—Section of gastrula of *Eupomatus*. Fixed in Flemming. $\times 600$.
 „ 6.—Section of a gastrula of *Eupomatus*. Fixed in Corrosive and acetic. $\times 600$.
 „ 7.—Section of *Eupomatus* egg. Fixed in Flemming. $\times 600$.
 „ 8.—Section of a gastrula of *Polygordius*. Fixed in Corrosive and acetic. $\times 600$.

*An Experimental Enquiry into the Factors which Determine the Growth and Activity of the Mammary Glands.**

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(From the Physiology Laboratory, University College, London.)

[PLATE 19.]

The correlation between the mammary glands and the other organs concerned in generation presents perhaps the most striking example of the interdependence of the growth and activities of different organs of the body. Although the manner in which this correlation is brought about has been the subject of speculation for many years, especially among medical men, it is only quite recently that any attempt has been made to apply experimental methods to its explanation.

In the case of the mammary glands we have organs which are present in both sexes, and at birth are in the same immature condition. In both sexes there is frequently, during the few days after birth, an enlargement of the glands and an actual secretion of fluid, which is known as “witch’s milk.” This state of activity disappears at the end of the first or second week, and till puberty the glands remain in the same undeveloped condition. At this period in Man the first difference appears between the mammary glands of the two sexes, a rapid growth taking place in the female, and accompanying the commencement of the ovarian functions.

During adult life there is apparently at each œstral period a slight and

* In this investigation the operations and inoculations were carried out by E. H. Starling, and the preparation of the extracts and the microscopic examination of the glands by J. Lane-Claypon.

506 Dr. J. E. Lane-Claypon and Prof. E. H. Starling. [Feb. 12,

temporary enlargement of the gland, and during the whole of adult life the glands remain in the female at the same state of development unless pregnancy occurs. The commencement of pregnancy acts as an impetus to a further great development of the gland substance, a development which, as we shall see shortly, differs in some respects from that which gave rise to the growth of the gland at puberty. This hypertrophy continues throughout the whole of pregnancy, being more rapid as pregnancy advances.

With cessation of pregnancy, whether abnormally or at the normal term, the growth of the glands at once comes to a stop, and two or three days after this cessation the activity which previously was spent on growth is now applied to the secretion of milk, a secretion which, if the gland is emptied periodically, lasts for many months.

This whole cycle of changes can be prevented by the removal of the sexual glands, namely, the ovaries, and these organs must therefore be regarded as primarily responsible for the growth of the mammary glands. We shall have to consider later on whether they are the immediate source of the impulses which induce the growth of the gland during pregnancy and its secretory activity after parturition. We must conclude, however, that there is some impulse or impulses arising in the ovaries, uterus, or product of conception, which exerts a direct influence on the mammary glands, and is responsible for their hypertrophy.

What is the nature of this connection? The well-known nervous connections between the mammary glands and the uterus, instanced by the occurrence of uterine contractions on stimulation of the nipples, has been taken to indicate that the impulses which cause growth of the glands are also nervous in character. Although this view is still sustained by certain physiologists, *e.g.*, K. Basch,* there are many facts, experimental as well as chemical, which place such an explanation out of court. Thus in a goat Eckhard† cut all the nerves to the mammary glands without appreciably affecting the yield of milk from the denervated gland. Ribbert‡ transplanted in the guinea-pig one of the two mammary glands to the neighbourhood of the ear, thus dividing all its nervous connections. In a subsequent pregnancy this gland enlarged as usual, and milk could be obtained from it at the end of pregnancy.

Still more definite evidence is afforded by the well-known case in which Goltz and Ewald§ removed the whole of the lumbo-sacral cord in a pregnant

* 'v. Ergebnisse der Physiologie,' vol. 2, 1, p. 325, *et seq.*, 1903; also Bibliography.

† 'Eckhard's Beiträge,' vol. 1, p. 18.

‡ 'Archiv f. Entwicklungsmechanik,' 1898.

§ 'Pflüger's Archiv,' vol. 63, p. 385.

1906.] *Growth and Activity of the Mammary Glands.* 507

bitch, thus severing all possible nervous connections between the pelvic organs and the mammary glands. This dog gave birth to puppies at full term. Pregnancy was attended with the normal hypertrophy of the mammary glands, and the bitch successfully suckled one of the puppies. A similar case has been recorded by Routh.*

A woman when seven months pregnant was the subject of fracture of the spine at the sixth dorsal vertebra, completely crushing the spinal cord at this level. Parturition occurred two months later, and the woman was able to suckle the child normally.

Moreover we have experimental evidence that the correlating mechanism between the different parts of the generative system is not nervous. Thus Knauer† showed that, whereas extirpation of both ovaries puts an end to the periodical changes in the uterus which are responsible for the phenomena of "heat," both ovaries can be transplanted, thus dividing all their nervous connections, without undergoing complete atrophy and without abolishing the phenomena of "heat." We must conclude therefore that the connecting link in all these cases is chemical rather than nervous. It remains for us to determine the origin of the exciting substance or substances, as well as their mode of action.

In the case of the mammary glands we have really two questions to decide. We have to determine why the secretion of milk appears only at the end or after the termination of pregnancy, and in the second place the origin of the stimulus which, during pregnancy, is responsible for the hypertrophy of the mammary glands.

The Causation of the Secretion of Milk after Parturition.

In Man the secretion of milk commences two to three days after parturition. During the greater part of pregnancy a fluid can be expressed from the breasts, and this fluid can be obtained in greater quantity after birth; it is not true milk, but a watery fluid rich in proteids, which is called colostrum. In certain recorded cases where pregnant women have suckled during pregnancy, the fluid that has been obtained has also not been true milk, but of the nature of colostrum. In some animals, such as the rabbit, true milk can be obtained from the glands during the last two days of pregnancy. In each case, however, the factor which sets the secretory process going must be the same, and the question at once arises whether the secretion is due to a change in the metabolism of the uterus or ovaries, or to the absorption from the involuting uterus of products which may act as a special stimulus to the hypertrophied gland.

* 'Obstetrical Society of London Trans.,' vol. 39, 1897.

† 'Archiv f. Gynäkol.,' p. 322, 1900.

508 Dr. J. E. Lane-Claypon and Prof. E. H. Starling. [Feb. 12,

Observations on Man show that the secretion of true milk cannot be due to a stimulus received by the gland at the time of conception, since the secretion occurs whenever pregnancy comes to an end, whether at the third month or at the ninth. A good account of the clinical evidence bearing on this point has been given by Halban.* This observer points out that when premature death of the foetus occurs without its expulsion, as in cases of extra-uterine foetation, the breasts swell two or three days after the death may be presumed to have taken place, and a true secretion of milk may be obtained.

The same thing occurs when the death of the foetus happens *in utero*, but the result is not invariable. Thus Halban mentions certain cases in which the secretion of milk began two or three days after the presumed death of the child, but he also gives details of other cases in which the secretion occurred only after the expulsion of the dead foetus. Halban is inclined to ascribe the difference between these two classes of cases to differences in the placenta, and imagines that the appearance of milk in the breasts is determined, not by the removal or the death of the living foetus, but by the removal or death of the placenta.

The hypothesis that some special stimulating substance is formed in the involuting uterus or in the ovaries after parturition seems to be negatived by certain cases in which Porro's operation was performed without interfering with the subsequent onset of lactation. In order, however, to decide this point we have carried out a number of observations on pregnant rabbits. In these the whole uterus, uterine appendages, and ovaries, were removed at different stages of pregnancy. In the rabbit pregnancy lasts, on the average, 28 to 30 days. If in a primiparous rabbit Porro's operation be performed at the 10th day or at any day before the 14th, the development of the mammary gland at once ceases and gives place to retrogression. No milk, however, appears in the gland, and at the end of a couple of months the gland is little removed from that of a virgin animal. If, on the other hand, the operation be carried out on animals after the 14th day of pregnancy, within two days after the operation milk can be expressed from the nipples, showing that here, as in Man, the effective factor in determining the process of lactation is not the absorption of substances from ovaries or involuting uterus, but the *removal* of certain stimuli which normally proceed from the organs or foetus of the pregnant animal.

The most obvious explanation of these results is that lactation is due to the removal of the stimulus which during pregnancy occasions the hypertrophy of the mammary gland. Hildebrandt† has suggested that during pregnancy

* 'Archiv f. Gynäkol,' vol. 72, Heft 2, 1905.

† 'Hofmeister's Beiträge,' vol. 5, p. 413, 1904.

1906.] *Growth and Activity of the Mammary Glands.* 509

an impulse is exerted by the developing ovum on the mammary glands which acts as a stimulus to growth, and at the same time protects the cells of the gland from those autolytic disintegrative processes which occur to a large extent in the secreting gland. That the act of secretion can be ascribed to autolytic processes of the gland seems highly improbable. We have no evidence that the autolysis of the gland cells would give rise to the specific constituents which characterise milk, but Hildebrandt's idea of an inhibitory substance which excites hypertrophy of the gland, and whose removal leads to secretion, is a valuable one.

Our whole idea of inhibition involves the stopping of the normal dissipative processes in a tissue and the augmentation of the assimilative, so that the continued reception of inhibitory impulses must lead to an actual increase in the substance of the cells, with resulting division and growth of the gland. But, as Hering has pointed out, the more this process of assimilation is carried on, the greater is the tendency to autonomous dissimulation, and the removal at the end of pregnancy of inhibitory impulses, or the attainment towards the end of pregnancy of a maximum degree of hypertrophy, must result in an autonomous dissimulation, *i.e.*, activity of the tissue. The normal activity of the mammary gland is secretion, and it is entirely in accordance with the accepted principles of physiology that the stimulus, which during pregnancy gave rise to growth, should by its removal at the end of pregnancy be responsible for the act of lactation.

We may conclude then that lactation is due not to excitation of the gland by special substances produced in the other generative organs, but to the removal of the stimulus which during pregnancy was responsible for the growth of the gland.

The Origin of the Substance or Substances by which the Hypertrophy of the Mammary Glands is determined.

We have seen reason for regarding the development of the mammary glands, which occurs in the female at puberty, as being due to the production of some substance in the ovaries which must reach these glands by way of the blood. It is probable that the slight increase in size of these glands at each œstral period is also determined by the greater activity of the ovaries at these periods, an activity which has been shown by Marshall* to be probably responsible for the changes which occur in the uterus. These changes are, however, insignificant as compared with the enormous hyperplasia which is associated with pregnancy. The question arises whether the greater growth during pregnancy is to be ascribed to an increased production of the specific

* 'Phil. Trans.,' B, vol. 198, 1905.

510 Dr. J. E. Lane-Claypon and Prof. E. H. Starling. [Feb. 12,

chemical excitant by the ovaries, or to the changed metabolism of the uterus during pregnancy, or whether the new products of conception, namely, the foetus and placenta, represent the seats for the manufacture of the mammary hormone.

There is a large amount of clinical evidence which enables us to decide with some probability, though without certainty, between these various alternatives. Conception is followed by a great increase in the size of the ovaries, chiefly in consequence of the formation of the *corpus luteum*. This growth has been associated by Born, Fränkel,* and Marshall† with the production of a specific excitant for the uterine mucous membrane which determines the growth of this membrane and the attachment of the embryo. These observers have shown that extirpation of both ovaries at an early period in pregnancy, or even destruction of the *corpora lutea* by means of the cautery, causes the cessation of the pregnancy. We ourselves have found that extirpation of both ovaries in the rabbit if carried out before the 15th day is apt to cause abortion. Later on in pregnancy oophorectomy can be performed without interfering with the course of the pregnancy.

The growth of the ovaries is, however, chiefly marked in the first third or half of pregnancy. After this time the *corpora lutea* begin to diminish in size, and with them the whole ovaries. On the other hand, the growth of the mammary gland, although marked almost immediately after impregnation, becomes more and more rapid with the advance in pregnancy, and the growth during the last half of pregnancy is many times greater than that during the first half. Moreover, numberless instances in man show that extirpation of both ovaries, as in double ovariectomy, does not necessarily interfere with the course of pregnancy or with the growth of the mammary glands. Labour may come on at the usual time, and be followed by normal lactation.

The fact that the mammary glands undergo normal hypertrophy in extra-uterine foetation, where the growth of the uterus is only a small fraction of that occurring under normal circumstances, seems to point to the products of conception, *i.e.*, either the foetus or placenta, or both, as the seats of origin of the mammary hormone. This conclusion is borne out by the fact that in extra-uterine foetation, if the death of the foetus occurs, this event is followed within a few days by the appearance of milk in the hypertrophied gland. The same result occurs when the death of the foetus happens *in utero* some time before its expulsion. On the other hand, as mentioned in the earlier part of this paper, cases have been recorded in which the death of the foetus

* 'Arch. f. Gyn.,' vol. 68, 1903.

† *Loc. cit.*

1906.] *Growth and Activity of the Mammary Glands.* 511

probably occurred a considerable time, a week or more, previous to its expulsion, and yet the appearance of milk in the gland has only taken place two or three days after the actual labour. Halban concludes, though, as it seems to us, on insufficient grounds, that the difference between the two sets of cases depends on whether the death of the placenta accompanies that of the foetus, and locates the seat of production of the specific hormone not in the foetus, but in the chorionic villi and placenta.

It is evident that no satisfactory solution of this question can be arrived at except by experiment. If any of the organs we have mentioned is the seat of production of a hormone which determines the growth of the mammary gland, it should be possible to obtain this hormone by extraction of the organ, and by its injection into a virgin animal produce the changes in the mammary glands which are characteristic of pregnancy. It is on these lines that we have proceeded in the present research, and have chosen rabbits as subjects of experiment since they can be obtained in large numbers, are easily handled, and breed fairly well in confinement. Before describing the experiments themselves we must say a few words as to the normal course of development of the mammary gland in this animal during pregnancy.

The Natural Growth of the Mammary Glands.

In a virgin rabbit of eight months to a year old, the average age of the rabbits used in our experiments, it is difficult with the naked eye to see any trace of the mammary gland in the tissue lying under the nipples. In order to bring the gland into view we have removed the skin with the subcutaneous tissue, dissected the latter away from the skin, stretched it on a ring of cork, and then, after hardening, stained it in a weak solution of hæmatoxylin. After staining, the subcutaneous muscle fibres were dissected away, leaving only connective tissue, with any mammary gland tissue which might be present, surrounding the nipple. In the spread-out specimen it is possible to see in the immediate neighbourhood of the nipple the ducts which form the mammary gland. As a rule they are limited to an area not more than 1 cm. broad. Plate 19, fig. 1, shows the largest extent of mammary gland we have ever observed in a virgin rabbit of this age. On section the gland is found to consist entirely of ducts, which are lined with a single layer of flattened epithelial cells, and terminate blindly. In no case is there any trace of the alveoli which are so characteristic of the fully-formed gland.

With the occurrence of conception a marked change begins in the gland. Four or five days after conception, when it is still impossible with the naked eye to discover any embryos in the swollen uterine horns, on reflecting the skin from the abdomen each mammary gland appears as a circular pink area,

512 Dr. J. E. Lane-Claypon and Prof. E. H. Starling. [Feb. 12,

about 2 to 3 cm. in diameter, surrounding the situation of each nipple (fig. 2). On section the gland is seen to consist still merely of ducts. These however are in an active state of proliferation, throwing out tree-like branches towards the periphery. The epithelial lining of the ducts is two or three cells thick; the cells are much more swollen than in the virgin gland, and numerous mitotic figures are seen in the epithelium. This growth proceeds rapidly, so that about the ninth day, on reflecting the skin from the abdomen, the whole is found to be covered with a thin layer of gland tissue. The margins of the glands are now almost contiguous, and the glands vary in diameter from 5 to 8 cm. (fig. 3). The appearance of the branching ducts in the stained specimen is also somewhat different, the ducts presenting, especially towards the periphery of the gland, cauliflower-like excrescences from their margins. The extreme periphery of the gland is generally somewhat thicker than the rest of the gland. On section it is found, especially at the periphery, that the formation of alveoli is commencing. This formation of alveoli proceeds henceforth rapidly, together with a continued growth of the ducts. At the twenty-fifth day the whole surface of the abdomen is covered with a layer of gland tissue, which may be 0.5 cm. thick, and on section is found to be formed for the greater part of alveoli. About this date, too, fat globules are forming in the cells of the alveoli. In the rabbit pregnancy lasts about 30 days. During the greater part of pregnancy, from the ninth day to the twenty-fifth, a watery fluid can be squeezed from the nipples. During the last two or three days of pregnancy this fluid becomes milky in character, so that immediately after parturition the mammary glands are already full of milk.

THE ARTIFICIAL PRODUCTION OF GROWTH IN THE MAMMARY GLANDS.

We may now proceed to an account of the experiments in which we tried to reproduce artificially some of the remarkable changes which are normally determined by pregnancy. It was evident to us, before we began our researches, that it would be difficult, if not impossible, to present any stimulus to the mammary glands which would be as effective as the normal one. For, wherever the mammary hormone is manufactured, the manufacture must be assumed to proceed continuously. There is therefore a constant leakage of the active substance into the blood, and it is probable that the amount of this substance produced increases with the duration of pregnancy. At no time will the mammary gland be set free from the influence of this specific stimulus. On the other hand, however, we might prepare our extracts of the tissues, we could not expect to get more than the amount residual in the tissue and caught, so to speak, in its progress through

1906.] *Growth and Activity of the Mammary Glands.* 513

the placenta into the maternal blood-vessels. This amount we might inject into our rabbits, but it would probably be taken up and absorbed into the circulation of the rabbit long before we were ready for our next injection, so that, whereas under normal circumstances the mammary glands are being continuously stimulated to hyperplasia, we could not expect to do more than give these glands a series of small shoves in the same direction.

Methods of Experiment.

Our object in the present research was to attempt to imitate the processes of pregnancy, by the injection of fluid extracts containing the soluble constituents of the various tissues, ovaries, uterus, placenta, or foetus into a virgin rabbit. The extracts were prepared in various ways. In some of our earlier experiments an emulsion of the tissue in normal salt solution, prepared with aseptic precautions, was injected subcutaneously. This method limited us to the use of only small portions of tissue. Since the other hormones with which we are acquainted, such as secretin or adrenalin, are soluble and diffusible substances, in later experiments we used a much greater mass of tissue for the preparation of fluid extracts, which were made sterile and freed from solid particles by passage through a Berkefeld filter. In all cases the tissues were ground up thoroughly with sand. The resulting paste was then in some cases mixed with normal salt solution, allowed to stand for two or three hours, and centrifuged. The supernatant fluid was passed through a Berkefeld filter, and the resulting clear filtrate used for injection. In other cases the ground-up tissues were mixed with Kieselguhr, and the juices expressed in a Büchner press; the press-juice thus obtained was passed through a Berkefeld filter before injection. The injections—which were naturally made with aseptic precautions—were in the early experiments subcutaneous. As, however, we increased the bulk of the injection, we adopted the plan of injecting into the peritoneal cavity. A further advantage of injecting into the peritoneal cavity is that one not only obtains quicker absorption, but is free from the disadvantage attendant on subcutaneous injection, namely that the fluid introduced under the skin of the back tends to flow down to the subcutaneous tissue in the abdomen, so that the mammary glands are bathed in an aseptic fluid containing proteids, which might in itself serve as a stimulus to proliferation.

Experiments on Injection of Tissue Extracts.

Our experiments can be divided into the following groups according to the organs injected, namely injection of ovaries, of uterine wall or mucous membrane, of placenta, of placenta plus uterus, of foetus, together with

514 Dr. J. E. Lane-Claypon and Prof. E. H. Starling. [Feb. 12,

placenta and membranes, or of the foetus alone. Finally, we made certain control experiments in which the animals were injected either with emulsion of liver or with blood serum obtained from normal animals.

I.—Ovaries.

Experiment 1. April 22, 1904.—Two ovaries from rabbit 15 days pregnant were implanted into peritoneum of second non-pregnant rabbit.

May 3.—Rabbit killed. No change in mammary glands; the implanted ovaries were necrosed and vascularised; uterus and vulva congested. On microscopic examination there was no proliferation of mammary gland. The uterine mucous membrane was typical of "heat."

The rabbit from which the ovaries were taken aborted immediately after the operation.

Experiment 2. May 31, 1904.—A rabbit which had been in the laboratory since January was injected with chloroform water extract of six ovaries from three rabbits, each about the fifteenth day of pregnancy. The rabbit received three injections in the course of nine days.

It was killed on June 13, four days after the last injection. No changes were found in the mammary glands, and there were no changes in the uterus.

Experiment 3. June 6, 1904.—A virgin rabbit was injected on six occasions during 19 days with an aseptic emulsion of 10 ovaries from rabbits between 11 and 14 days pregnant.

June 20.—Killed. Mammary glands invisible, uterus enlarged and congested, with proliferation of mucous membrane.

Experiment 4. May 16, 1904. Virgin rabbit received 13 injections of the saline extract of 26 ovaries between May 16 and June 1.

June 1.—Killed. The uterus slightly congested. No effect on mammary glands.

II.—Uterus and Uterine Mucous Membrane.

Experiment 5. May 30, 1904.—Virgin rabbit, about six months old, injected with chloroform water extract of the mucous membrane of four pregnant uteri. During 14 days it received four injections.

It was killed on June 20. No changes, either of mammary glands or of uterus.

Experiment 6. October 12, 1904.—Two virgin rabbits received during a period of 19 days seven injections of the filtered saline extract of the mucous membrane of seven pregnant uteri. One rabbit (A) received the boiled extract, the other rabbit (B) received the unboiled extract. Rabbit A was killed on October 31. The uterus and ovaries were congested (the animal was in heat), but there was no growth of the mammary glands.

Rabbit B, killed on November 2. The mammary glands showed no change, but the uterus in this one was also congested.

Two other experiments were made on the injection of uterine mucous membrane, but with no result.

III.—Injection of Placenta.

Experiment 7. May 15, 1904.—A virgin rabbit received the unboiled extracts (press juice) of the mucous membrane of 14 pregnant uteri and 123 placentae. These were given in 14 injections between May 15 and June 1. It was killed on June 2. The mammary glands were unaltered. The uterus was congested.

Experiment 8. October 12, 1904.—Two virgin rabbits, A and B.

A received on eight occasions during 19 days the boiled extract of 55 placentae. It was killed on October 31. No results either on uterus or mammary glands.

1906.] *Growth and Activity of the Mammary Glands.* 515

B received the unboiled extract of 55 placentæ. It was killed on November 2. Mammary glands unchanged ; uterus slightly congested.

IV.—*Injection of Fœtus with or without Placenta and Uterine Mucous Membrane.*

The first three experiments on this point were also fruitless so far as regards any change in the mammary glands. In these cases the rabbits received from five to seven injections of extract of fœtus. The following experiments, therefore, were conducted for a longer period of time, and a much larger number of injections were given, and in these positive results were obtained.

Experiment 9.—February 8, 1905.—A rabbit, eight months old, which had been in the laboratory since it was a fortnight old—virgin—received, *subcutaneously*, extracts made by rubbing up fœtuses and their placentæ with sand, extracting with normal saline, centrifuging, and filtering through a Berkefeld. The injections were made as follows :—

February	8.	—	Half the extract of 7 fœtuses at the 20th day.
"	9.	—	" " 7 " 20th "
"	10.	—	4 fœtuses at the 22nd day.
"	11.	—	5 " 14th "
"	13.	—	4 " 14th "
"	14.	—	3 " 22nd "
"	15.	—	4 " 20th "
"	16.	—	Half the extract of 8 fœtuses at the 20th day.
"	17.	—	" " 8 " 20th "
"	18.	—	6 fœtuses at the 19th day.
"	20.	—	5 " 14th "
"	21.	—	Half extract of viscera of 6 fœtuses at the 25th day.
"	22.	—	" " 6 " 25th "
"	23.	—	Viscera of 7 fœtuses at the 20th day.
"	24.	—	" 4 " 24th "

The rabbit was killed on February 25. There was marked hypertrophy of the mammary glands, which were surrounded with a certain amount of fluid remaining from the subcutaneous injections. The ovaries were large, the uterus congested, and the vulva showed signs of heat. On microscopic examination there was found to be marked hypertrophy of ducts, which were branching and lined with many layers of cells. The mitotic figures were numerous. Specimens of the mammary glands from this rabbit were shown at the meeting of the Physiological Society at University College in March, 1905.

Experiment 10. February 27, 1905.—Intraperitoneal injection of press-juice of the viscera of fœtuses, together with placentæ and uterine mucous membrane.

A virgin rabbit received the following injections :—

February	27.	—	8 fœtuses, etc., at 24th day.
"	28.	—	Half of 9 fœtuses, etc., at 24th day.
March	1.	—	" 9 " 24th "
"	2.	—	8 fœtuses, etc., at 25th day.
"	3.	—	8 " "
"	4.	—	2 " "
"	6.	—	6 " "
"	7.	—	4 " "

516 Dr. J. E. Lane-Claypon and Prof. E. H. Starling. [Feb. '12,

Interval of 10 days, owing to failure of supply of pregnant animals.

March	17.—	7	fœtuses, etc.,	at 16th day.
"	21.—	5	"	26th "
"	23.—	8	"	22nd "
"	24.—	14	"	14th "
"	25.—	12	"	20th "
"	27.—	5	"	20th "
"	28.—	8	"	22nd "
"	29.—	5	"	21st "
"	30.—	7	"	17th "
"	31.—	4	"	20th "
April	1.—	9	"	13th "
"	3.—	9	"	18th "
"	5.—	5	"	15th "
"	6.—	11	"	16th "
"	7.—	6	"	18th "

This rabbit, therefore, received the fluid extracts of the viscera of 160 fœtuses. It was killed on April 8. On reflecting the skin of the abdomen, the mammary glands were seen to be markedly hypertrophied. The margins were almost contiguous, and were somewhat raised and pink, presenting, therefore, much the appearance which is seen in a pregnant rabbit of the eighth or ninth day. The general aspect of the stained gland is shown in fig. 4. On microscopic section not only was there marked duct proliferation with mitotic figures, but at the thickened border the formation of alveoli was just commencing. The whole of these injections had been made intra-peritoneally, or, on one or two occasions, under the skin of the legs, so that there was no infiltration of the connective tissue surrounding the mammary glands. This is the best result which we obtained.

Experiment 11. May 2 to June 1, 1905.—In this experiment we sought to determine whether the growth-producing substance is contained chiefly in the viscera or in the body, *i.e.*, muscles, skin, and bones, of the fœtus, and, moreover, whether it could be extracted from these tissues by boiling. Unfortunately, however, out of the four rabbits which we chose for this experiment, only two were definitely virgin, so that the results in the other two cases were equivocal.

Rabbit 1, not a virgin, received the pressed juice of the viscera.

Rabbit 2, a virgin, received the filtered boiled extract of viscera.

Rabbit 3, also a virgin, received the pressed juice of the bodies of the fœtuses unboiled.

Rabbit 4, which was evidently multiparous, received the boiled extract of the bodies of fœtuses. From this rabbit, at the commencement of the experiment, a small portion of mammary gland was taken as a control.

All four rabbits received portions of 182 fœtuses of all ages between May 2 and May 31.

From Rabbits 1 and 4 milky fluid could be expressed from the nipples after the ninth injection.

In Rabbit 2 a watery fluid could be expressed from the nipples after the seventeenth injection.

Rabbit 3 showed traces of watery secretion after the twelfth injection.

All four were killed on June 1. Results were as follows:—

Rabbit 1.—Multiparous. Mammary glands well developed and showing many alveoli on microscopic section. The ducts were full of milk; they were, however, lined with only a single layer of epithelium, and it was impossible to say that any hypertrophy had taken place.

Rabbit 2.—Virgin. The mammary glands did not present much enlargement as judged from inspection. On microscopic examination, however, many branching ducts were

1906.] *Growth and Activity of the Mammary Glands.* 517

observed lined with two layers of cells, presenting the same appearance, but in a smaller degree, as those in the glands of the rabbit in Experiment 10.

Rabbit 3.—Virgin. Glands large, hypertrophied, containing a fair amount of watery fluid. Alveoli present and ducts showing proliferation.

Rabbit 4.—Not virgin. Mammary glands fully marked, and distended with milky fluid, but impossible to determine whether or not hypertrophied.

In order to be certain of the induction of growth in the mammary gland by the injection of extracts of foetus, three more experiments were made. In the first of these, in which the rabbit received 16 injections of the pressed juice of the viscera of 138 fetuses, the results were absolutely negative. In this experiment, however, we had been obtaining very small amounts of pressed juice from the tissues, and we thought that the absence of result might possibly be due either to retention of the active substance by the Kieselguhr or to insufficient destruction of the cells in the process of grinding. It is possible, too, that immaturity of the rabbit may have been in some measure responsible for the negative result.

In the next two experiments, therefore, we abandoned the Büchner method and, after grinding with sand and with normal salt solution, centrifuged and filtered the supernatant liquid through a Berkefeld candle before injection. Both these experiments gave positive results.

Experiment 12.—October 4 to 21. Virgin rabbit, full-grown. Received daily, intraperitoneally, the saline extract of a number of fetuses about the fifteenth to twentieth day of pregnancy. Killed on the 21st. It showed distinct growth of the mammary glands with duct proliferation (*vide* fig. 5).

Experiment 13.—October 4 to 21. Virgin rabbit. Received the saline extract, intraperitoneally, of the bodies and placenta of the same fetuses used in Experiment 12. Fifteen injections were given in the 17 days. Killed on the 21st. It showed marked growth of mammary glands with plentiful mitotic figures. The appearance of this gland in the stained specimen is shown in fig. 6.

Discussion of Results.

From the results just described, it will be seen that in six cases we succeeded in producing in virgin rabbits a growth of mammary glands similar to that occurring during the early stages of pregnancy, and consisting in the proliferation of the epithelium lining the ducts, with the multiplication of these ducts by branching into the surrounding tissues. In one of these (Experiment 10) where our injections were carried out during five weeks and the experiment lasted nearly seven weeks, there was an actual formation towards the periphery of the gland of secreting acini. In some of these cases, however, namely those in which the injections had been given under the skin of the back (*e.g.*, Experiment 9), the mammary glands were bathed for considerable periods of time in the injection, and it seemed to us possible that this might be a determining factor in producing growth.

We therefore carried out a control experiment on a virgin rabbit, in which normal rabbit's serum was injected, for the most part subcutaneously, for a period of three weeks. The serum, which was derived from non-pregnant animals, but contained much more nutrient material, *e.g.*, proteid, than the

518 Dr. J. E. Lane-Claypon and Prof. E. H. Starling. [Feb. 12,

fluids used in our previous injections, ran down in the subcutaneous tissue, so that during the whole duration of the experiment the abdominal wall was thickened and œdematous through the presence of the serum. On killing the animal at the end of three weeks the glands were little, if any, larger than those usually obtained from a virgin animal (fig. 7). On section, however, mitoses were present in the epithelium of the ducts, and there was apparently a certain amount of proliferation of the ducts.

We must conclude, therefore, that superabundant supply of nutrient material in the fluid surrounding the acini may lead to proliferation resembling in kind that which was produced by our injections. This result had not been produced in an earlier control experiment, in which we injected the saline extract of liver, and in view of the small results produced by the injection of the serum as compared with those produced by the injection of the extracts of foetus much poorer in proteids, we are inclined to believe that it is impossible to explain our results in the other experiments as due to the infiltration of the tissues round the glands. This explanation, at any rate, could not hold for the growth in Experiment 10, in which there had been at no time any injection into the subcutaneous tissue of the back. It is interesting, from the general pathological point of view, to note that typical epithelial proliferation in the ducts can be produced by an abnormally large supply of proteid in their surrounding lymph, and the subject is worthy of further investigation.

A striking fact in all our experiments with a positive result is the smallness of the growth produced as compared with the quantity of material used for injection. In all the positive cases the material for injection was derived from foetuses; in Experiments 11 (2) and 12 from the viscera only; in Experiment 11 (3) from the bodies only; in Experiments 9 and 13 from the foetuses together with placenta; and in Experiment 10 from the foetuses, placenta, and mucous membrane of uterus together. On the other hand, injection of extracts made from ovaries, uterus, or placenta alone had no effect on the growth of the gland. We are therefore justified in concluding that under normal circumstances the hormone which is responsible for the growth of the mammary gland during pregnancy is produced mainly in the growing embryo. This hormone, however, must be produced in minimal quantities. It is apparently not stored up in any of the tissues of the foetus or of the placenta, so that, in injecting extracts of foetus, we are simply injecting the small amount of material which is diffused through the juices on its way to the blood-vessels and into the maternal blood.

It is possible, of course, that the specific mammary hormone is produced from a precursor or mother-substance in some organ or other, and that future

research may reveal some method of splitting off the hormone in large quantities, and also of determining whether its production is diffused throughout all the tissues or is confined to one special organ of the body. Injection of extract of duodenal mucous membrane, for example, would give only minimal effects on the pancreas. We should not be justified in concluding from this absence of result that the duodenum was not the seat of origin of the chemical stimulus to the pancreas. Its peculiar relation to the pancreas is only brought into prominence when it is treated with acid, so as to liberate the secretin from its mother substance.

Our experiments, therefore, throw no light on the seat of production of the hormone in the foetus. Apparently the extent of the growth obtained is a function of the quantity of tissue used in preparing the extracts. The widespread occurrence of the substance in the body of the foetus points to its being extremely diffusible, as indeed we should expect from analogy with other hormones.

We can only say, therefore, that the hormone is produced by some or all the tissues of the fertilised ovum, whence it is carried off by the blood to the placenta, and so makes its way by diffusion into the maternal blood-vessels. Whether it is identical with the substances which are responsible for the production of the other changes associated with pregnancy, or whether there are distinct substances acting on each organ which is modified during this condition, our experiments do not show. But we have evidence that in the foetus itself the hormone or hormones of pregnancy have the same result as in the maternal organism. Thus there is increased growth of the mammary glands in the foetus during the last month of pregnancy, and also in the female an increase in the uterine mucous membrane, as has been shown by Halban. After birth the mammary glands may begin to secrete just as after pregnancy, and there are changes in the uterine mucous membrane similar to those associated with menstruation.

Are we to regard, then, the foetus as the only source of this hormone? The facts mentioned at the beginning of this paper show that such a conclusion is impossible. The growth of the mammary glands which occurs at puberty can only be ascribed to ovarian influence, and is absent if the ovaries have been previously removed, and Halban ascribes to this ovarian substance both the growth of the mucous membrane during each pro-oestrus and the swelling of the glands at each oestral period, which may in rare cases be attended or followed by the actual formation of milk. Halban explains in the same way those cases recorded by Heape and Kehrler, in which bitches, which had not been impregnated at the normal time, have, after two months, not only made a bed for their young, but have had swelling of the mammary

520 Dr. J. E. Lane-Claypon and Prof. E. H. Starling. [Feb. 12,

glands, with, in some cases, actual secretion of milk. He would regard this condition as being a continuance of the state of pro-œstrus leading to continued growth of mucous membrane and also of the mammary gland. When the impregnation was no longer possible, with the discharge of the ovum, the secretion of this substance ceased, and the absence of the inhibitory stimulus caused break-down of the uterine mucous membrane as well as dissimilative activity of the mammary gland.

During sexual life, therefore, the ovaries are continually producing a substance which exerts an influence on both glands and uterus. With the occurrence of conception there is at once a great growth of what we may call germinal material. With the growth of the fertilised ovum the amount of hormone produced in the ovum must also increase in proportion. In the early stages of pregnancy the chief source of this hormone may perhaps be located in the chorionic villi, but with the growth of the body of the foetus this latter must take a preponderating share in the preparation of the hormone. We have no reason to suppose that the foetal elements of the placenta entirely lose this function of the germinal cells, but the negative results of injection of placenta in our experiments show that it is impossible to ascribe to the placenta, as is done by Halban, a preponderating part in the preparation of this hormone.

If the hormone is produced in the body of the foetus, it might be objected that the formation should go on after birth, and therefore lead in the new-born animal to a continuance of the growth both of the mammary gland and of uterus. The profound changes in the environment of the new animal which occur at birth must, however, induce equally profound changes in its metabolism, and there is no difficulty in imagining that with the assumption of extra-uterine life the formation of this substance in the foetus comes to an end.*

The occurrence of growth in the mammary gland of a virgin rabbit and of secretion in the mammary gland of a multiparous rabbit from the injection of boiled extracts of foetus, seems to indicate that the specific hormone, like adrenalin or secretin, is not destroyed by boiling. Further evidence,

* In the ornithorhynchus pregnancy is associated with the growth of mammary glands, although the embryo in this animal is contained in an egg, and does not enter into any anatomical connection with the uterine wall. Halban points out, however, that the shell of the egg is porous, and that during its stay in the uterine cavity it increases in size, and the contained embryo grows, in consequence of the absorption of nutrient material from the fluid contained in the uterine cavity. If the embryo is able to absorb nutrient material from the uterine contents, it is equally able to give up to these contents diffusible substances, which may be taken up by the mucous membrane and carried by the circulation to the mammary glands. The condition in the ornithorhynchus cannot therefore be regarded as a disproof of the chemical theory which we have adopted throughout this investigation.

1906.] *Growth and Activity of the Mammary Glands.* 521

however, is required on this point, as also on the question whether the substance is specific to the animal, or whether the injection of extracts of the foetus of one animal would produce a growth of the mammary glands in another species. One experiment, in which we fed a kitten for three weeks on the foetuses of rabbits, was negative in its results. This might, however, have been due to the failure of the intestine to absorb the hormone without destruction, or to the failure of the immature glands to react to the minute stimulus which they received. So far as we know, secretin is not absorbed into the circulation when introduced into the stomach or intestine, and colossal doses of adrenalin have to be given by the mouth in order to produce any systemic effects.

The effect of the injections of foetal extracts on multiparous rabbits deserves some further mention. The multiparous rabbit differs from a virgin rabbit in possessing ready-formed alveoli, *i.e.*, secretory structures. On the theory which we have adopted, the circulation of the mammary hormone should diminish any secretion in these alveoli and should cause growth. In all our experiments at least 24 hours elapsed between each two injections. It is probable that the hormone was rapidly absorbed from the injection, and was therefore present in the blood of the animal only for a certain fraction, say a few hours, out of the 24. While it was circulating it should cause building up of the secreting cells. Directly, however, it ceased to circulate, the cells would enter into dissimilative activity resulting in secretion. By our injections, therefore, we are not able to imitate the continuous stimulus of pregnancy. We are rather producing each day a pregnancy of a few hours followed by a parturition. These factors should therefore result in the production of milk in any animals possessing the structures (*i.e.*, the alveoli), which are capable of secreting milk, and would therefore account for the secretion of milk observed by us in all the cases where multiparous rabbits were the object of our experiment.

CONCLUSIONS.

So far as our experiments go, they show that the growth of the mammary glands during pregnancy is due to the action of a specific chemical stimulus produced in the fertilised ovum. The amount of this substance increases with the growth of the foetus, and is therefore largest during the latter half of pregnancy. Lactation is due to the removal of this substance, which must therefore be regarded as exerting an inhibitory influence on the gland cells, hindering their secretory activity and furthering their growth. It is probable that the specific substance is diffusible, and will withstand the boiling temperature.

We cannot, however, claim that these conclusions of ours are firmly established. A final decision can only be given by a research carried on under more favourable conditions. One requires, in fact, a farm, where we could have at our disposal 500 rabbits, and could arrange for a plentiful supply each day of rabbits about the middle of pregnancy. Under these conditions it might be possible to determine both the seat and nature of the effective stimulus, as well as to test the influence of various reagents in splitting off the hormone from some possible precursor. Many of our experiments, carried out in a London laboratory, were brought to a premature conclusion by failure of material. If, however, the conception of the action of the mammary hormone, which was put forward by Hildebrandt and adopted by us, is correct, namely, that it is a substance which produces growth by inhibiting the normal activity of the gland cell, it should be possible to decide many questions affecting it by working on an animal, such as the goat, in lactation. Injection of the hormone should diminish or stop the secretion of milk while it was circulating in the blood, but should, as a secondary effect, produce an increased secretion as a reaction from the immediate assimilatory effect. The injection might, indeed, have to be prolonged for one or two days, since we know that in Man the onset of a renewed pregnancy during lactation stops the flow of milk only after some time (three or four weeks). At any rate, such experiments could be more rapidly carried out than those which have been the subject of this communication.

DESCRIPTION OF PLATE.

The drawings were made as follows:—The mammary glands were dissected out, pinned on corked rings, hardened in corrosive sublimate and formol, washed, and stained in very dilute hæmatoxylin. They were then dehydrated, cleared, and mounted as lantern slides in canada balsam between glass plates. (These specimens were shown by projection at the meeting of the Royal Society, on March 1, 1906.) An image of the specimens was thrown (without magnification) on to a piece of millboard, and the darkly stained glands were traced out in indian ink. The figures, therefore, reproduce the glands in natural size.

FIG. 1.—Gland from virgin rabbit.

- „ 2.—Mammary gland from primiparous rabbit, five days after impregnation.
 - „ 3.—Mammary gland from primiparous rabbit, nine days after impregnation.
 - „ 4.—Mammary gland from virgin rabbit which had received injections of extracts of foetuses, uterus, and placenta during five weeks (Exp. 10).
 - „ 5.—Mammary gland of virgin rabbit, showing growth produced by injection of extracts of foetal viscera during a period of 17 days.
 - „ 6.—Mammary gland of virgin rabbit, showing growth produced by injection of extracts of foetal bodies and placenta over 17 days.
 - „ 7.—Mammary gland of virgin rabbit, showing slight growth induced by daily subcutaneous injection of rabbit's serum (from non-pregnant rabbits) during a period of three weeks.
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Lane-Claypon and Starling. Roy. Soc. Proc., B. vol. 77, Plate 19.



FIG. 1.



FIG. 2.



FIG. 3.



FIG. 4.



FIG. 5.



FIG. 6.



FIG. 7.